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Residual Host Cell DNA Sample Preparation Kit (Fast) User Guide

Version: A/0

Product No.: 1104201

Reagents for 100 Extractions

For Research Use Only. Not for diagnostic or therapeutic use.

SHENTEK® Residual Host Cell DNA Sample Preparation Kit (Fast)

(Magnetic Bead Method)

Product No.: 1104201

Reagents for 100 Extractions

⚠ **WARNING!** Read this user guide thoroughly before starting the experiment. Pay close attention to the precautions and frequently asked questions.

⚠ **WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, mask, clothing and gloves.

Product information

Product description

The **SHENTEK® Residual Host Cell DNA Sample Preparation Kit (Fast)** is specifically designed for rapid pretreatment of host cell residual DNA samples in biological products and process intermediates. By combining advanced magnetic particles technology with an optimized lysis system, this kit ensures a rapid and efficient isolation of trace levels of host cell residual DNA from various matrices, including samples those containing interfering components such as chondroitin sulfate or heparin. Finally delivers high-quality samples for the downstream quantitative DNA analysis.

When combined with the rHCDpurify® system, this kit provides an automatic sample pretreatment workflow allowing convenient operations for automated lysis and nucleic acid extraction with a one-click start. It also supports manual extraction operations, providing flexibility to meet various needs from different laboratories. The purified extracts can be completely compatible with the SHENTEK® residual host cell DNA detection kit series, ensuring a robust performance in subsequent testing.

Kit components and storage

No.	Reagent	Part No.	Volume	Storage
I	Wash buffer A	NND014	1 × 30 mL	room temperature
	Lysis buffer I	NND102	1 × 20 mL	room temperature
	Lysis buffer II	NND103	1 × 60 mL	room temperature
	Elution buffer	NND018	1 × 10 mL	room temperature
	Dilution buffer	NND021	1 × 10 mL	room temperature
II	Magnetic particles	NND101	3 × 1 mL	2-8°C
III	Proteinase K	NND023	4 × 500 µL	-20°C*

*-30°C to -10°C is acceptable in accordance with USP <659>. Up to 24 months storage under proper conditions. Check the label for expiry date.

Materials required

Compatible Instruments (including but not limited to)	rHCDpurify® system or Magnetic stand
	Thermo Scientific KingFisher Flex
Equipments	Benchtop microcentrifuge
	Vortex mixer
	Dry bath incubator
	Pipettes
	Laminar flow cabinet
Consumables	Anhydrous Ethanol (AR grade)
	Low retention, sterile filter tips: 1000 µL, 100 µL and 10 µL
	Low retention, RNase/DNase-free, sterile microcentrifuge tubes, 1.5 mL
	96 deep well plate and plastic sleeve (Product No.: 1104192, if required)
	KingFisher 96 Deep-well Plate, (50 – 1,000 µL) (if required)
	KingFisher 96 Tip Comb（if required）

Workflow

Note: *The workflow described above should be adjusted and verified according to the actual application and operating conditions.*

Methods

Experiment preparation

Before first use of the kit:

- Add 40 mL of Anhydrous Ethanol to Wash buffer A (NND014) and mix thoroughly.
- Prepare a 70% (v/v) ethanol solution using ultrapure water and anhydrous ethanol, label it as "Wash Buffer B.
- Store Wash buffer A & Wash buffer B at room temperature properly to prevent evaporation.

Before each use of the kit:

- Preheat the dry bath to 55°C and 70°C (for manual procedure only).
- Remove the Magnetic particles (NND101) from the refrigerator at 2-8℃, mix thoroughly using a vortex mixer for 5-10 seconds, centrifuge briefly and keep at room temperature for at least 10 minutes to equilibrate the particles.

Sample pretreatment

1. Sample Dilution:

① If the test sample is an upstream purification process intermediate of a biological product, the residual DNA content is expected to be relatively high. To ensure the accuracy of the results, the measured values should fall within the linear range of the standard curve.

② If the sample is in dry powder form, ensure that it can be completely dissolved within the Dilution buffer (NND021) or a suitable alternative dilution buffer before proceeding to the next step. Dry powder sample are recommended to be reconstituted to a concentration range of 10 - 100 mg/mL.

2. Sample Premix Preparation:

Add 200 µL Lysis buffer I (NND102) + 20 µL Proteinase K (NND023) + 100 µL test sample to a 1.5 mL low-retention sterile microcentrifuge tube. Mix thoroughly by vortexing, then centrifuge briefly for 3-5 seconds.

Note: *For samples with low protein concentration or no detectable protein, the Proteinase K may be omitted; To ensure the accuracy of results, we recommend to perform triplicate DNA extraction for each sample.*

3. Extraction Recovery Control (ERC) Preparation:

Open one tube of the premixed sample solution prepared above. Based on the measured concentration of test sample, add a appropriate amount of standard material to prepare the ERC sample.

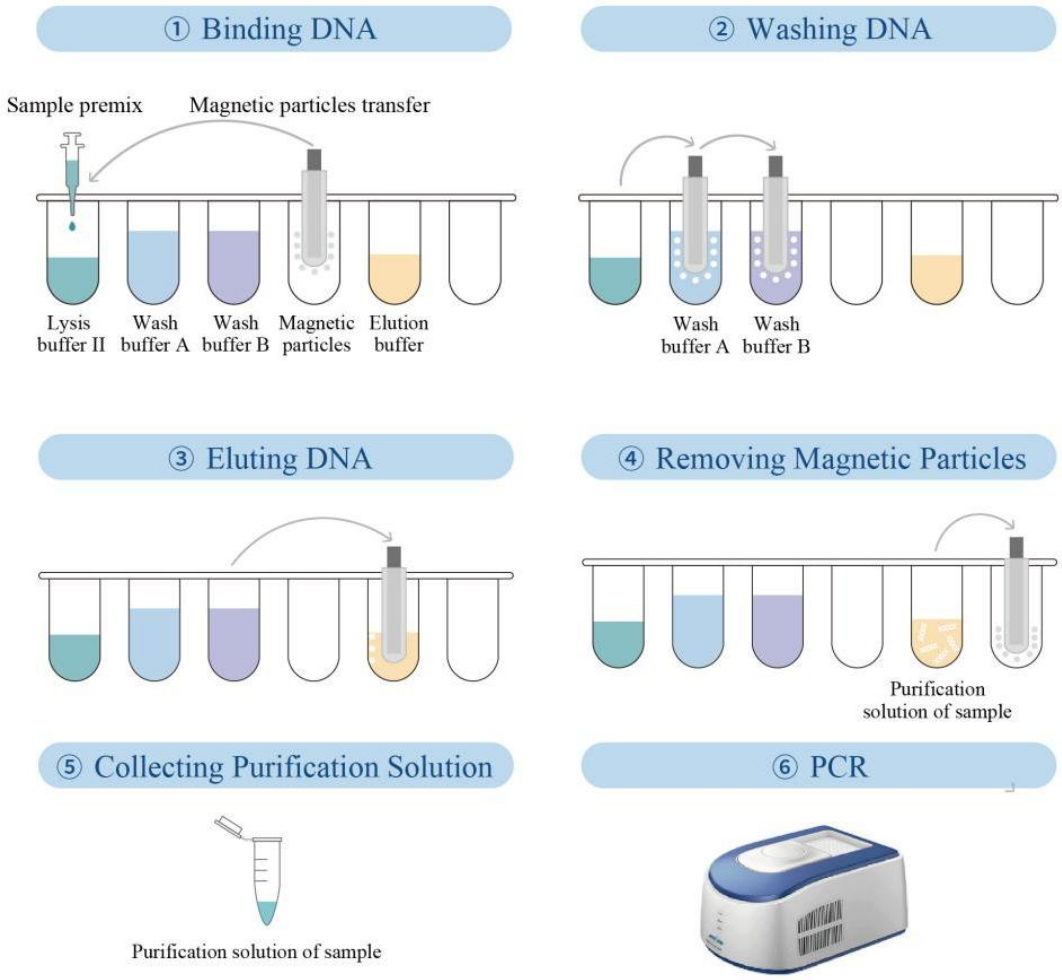
Note: *ERC is used to evaluate the DNA extraction recovery, accuracy and the overall performance of assay validation and reaction conditions. The recommend ERC concentration is 2-10 times that of the unspiked sample.*

4. Negative Control Sample (NCS) Preparation:

Each experiment shall include the NCS, prepared in the same procedure as unknown test samples, to evaluate whether there is cross-contamination or environmental contamination during sample processing.

DNA extraction

✧ Automatic extraction procedure with rHCDpurify® system



Extraction Preparation

Add the corresponding solution to each well according to the 96 deep-well plate layouts below:

Table 1. 96 deep well plate layouts

Group 1						Group 2					
1	2	3	4	5	6	7	8	9	10	11	12
S1						S1-ERC					
S2						S2-ERC					
S3						S3-ERC					
S4						S4-ERC					
S5						S5-ERC					
S6						S6-ERC					
NCS											

Column 1 or 7: Sample premix (200 µL Lysis buffer I + 20 µL Proteinase K+ 100µL test sample or other volume of ERC) + 600 µL Lysis buffer II to each well.

Note: *Transfer the entire sample premix to the corresponding wells of the 96 deep-well plate using low retention filter tips.*

Column 2 or 8: Wash buffer A 700 μL/well
Column 3 or 9: Wash buffer B 700 μL/well
Column 4 or 10: Magnetic particles 30 μL/well

Note: *Magnetic particles should be fully mixed in the vortex mixer for 5 seconds before use. If multiple samples are processed, thoroughly vortex the magnetic particles before each pipetting step to ensure consistency.*

Column 5 or 11: Elution buffer 100 μL/well.

Program Processing

1. Power button on → select "login" to enter account and password → enter the main page.
2. Wipe the interior of the instrument with a 75% ethanol → select "UV lamp" → set up "15 minutes".

   Note: *This step should be performed before the extraction preparation operation.*
3. Place the sample 96 deep well plate in a fixed position in the instrument and insert the plastic sleeve into the corresponding position.
4. Select "Run" → select “rHCD-201” program → scan the kit barcode → program running.
5. At the end of the program, a "drip" sound is emitted. Immediately remove the deep well plate and transfer all the purified sample solutions to a new 1.5 mL microcentrifuge tube.

✧ Automated extraction procedure with Thermo Scientific KingFisher Flex

Plate preparation

1. Before use, wipe the interior of the instrument with 75% ethanol. If required, perform UV decontamination with an external UV lamp for 15 minutes.
2. Prepare the reaction system according to the table below and label each plate:

Table 2. Example of 96-well Plate layouts

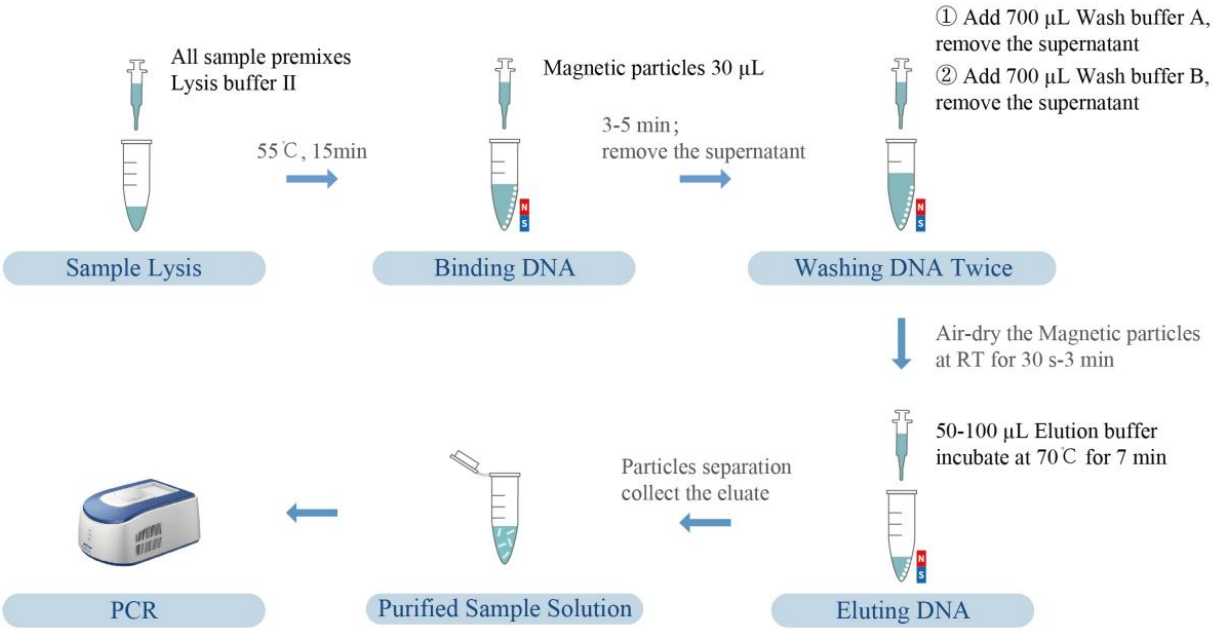
Plate ID	Plate position	Reagent	Volume per well
Sample and tips	1	Sample premix	200 μL Lysis buffer I + 20 μL Proteinase K + 100 μL test sample or other volume of ERC
		Lysis buffer II	600 μL
Beads	2	Magnetic particles	30 μL
Wash A	3	Washing buffer A	700 μL
Wash B	4	Washing buffer B	700 μL
Elution	5	Elution buffer	100 μL

Automated Instrument Procedure

1. Power on the instrument. It will perform self-check automatically and enter the protocol interface.
2. On the protocol interface, select “**ResDNA Sample Preparation Kit (Fast) for KingFisher Flex.bdz**” using the arrow keys, then select “Start” to launch the protocol.
3. Insert the Elution Plate as prompted, then select “Start” to confirm.
4. Insert Wash Plate B → select “Start”; insert Wash Plate A → select “Start”; insert Beads Plate → select “Start”.
5. Insert the Sample Plate with pre-loaded tip combs, then select “Start” to run the program.
6. The entire program runs for approximately 50 minutes.

   Note: *A1 corner at top-right for each plate.*

✧ Manual Extraction procedure



DNA Binding

For each sample tube:

1. Add all sample premixes (200 μL Lysis buffer I + 20 μL Proteinase K + 100 μL test sample or appropriate volume of ERC) with 600 μL Lysis buffer II, and vortex the tubes strongly to mix well. Spin briefly for 10 seconds in a microcentrifuge, then incubate at 55℃ in a dry bath for 15 minutes.
2. Spin briefly for 10 seconds in a microcentrifuge, then add 30 μL of Magnetic particles.

Note: *Magnetic particles should be fully mixed in the vortex mixer for 5 seconds before use. If multiple samples are processed, thoroughly vortex the magnetic particles before each pipetting step to ensure consistency.*

3. Vortex the tubes at high speed for 5 minutes to evenly distribute the magnetic particles and bind the nucleic acids. Centrifuge briefly for 10 seconds. Place the tubes on the magnetic stand with magnetic particles facing the magnet.
4. Allow the solution to become completely clear and the magnetic particles to be fully collected (approximately 3 - 5 minutes). Carefully remove the supernatant without disturbing the magnetic particles.

DNA Washing

For each tube with magnetic particles:

1. Add 700 μL of Wash buffer A, vortex for 1 minute to mix thoroughly then spin down for 10 seconds. Place the tubes on the magnetic stand until the particles are completely collected. Carefully remove the supernatant without disturbing the magnetic particles.
2. Add 700 μL of Wash buffer B, vortex for 1 minute to mix thoroughly then spin down for 10 seconds. Place the tubes on the magnetic stand until the particles are completely collected. Carefully remove the supernatant without disturbing the magnetic particles.
3. To completely remove the supernatant, an additional 10-second spin down and magnetic particles collection is recommended. Carefully remove any remaining liquid using a 10 μL pipette.

Note: *When removing the supernatant, avoid any aspiration of the magnetic particles.*

4. Keep the tube open, dry the magnetic particles at room temperature for 30 seconds - 3 minutes to evaporate any residual ethanol.

Note: *The drying time depends on the specific environment. It could be shorter in higher temperature or lower humidity conditions, while slightly longer in lower temperature or higher humidity conditions.*

DNA Eluting

For each sample:

1. Add 50 - 100 μL of pre-warmed (70℃) Elution buffer along the wall of centrifuge tube. Vortex for 5 seconds, then incubate at 70℃ for 7 minutes. Vortex 2 - 3 times during incubation to ensure complete resuspension.

Note: *Vortex the Magnetic particles and Elution buffer thoroughly. Briefly spin down the tube again to collect any magnetic particles and elution buffer remaining on the tube cap or wall.*

2. After incubation, spin down for 1 minute, and then place the tubes on the magnetic stand. Wait until the magnetic particles are completely collected, carefully transfer the eluate to a new microcentrifuge tube.
3. Briefly spin down the tubes from the previous step for 10 seconds, then place them on the magnetic stand again. Carefully transfer the eluate to a new 1.5 mL microcentrifuge tube to obtain the purified sample solution.

Note: *To ensure the accuracy of the test results, we recommend performing the sample purification and subsequent DNA testing on the same day.*

Precautions

1. Before starting the experiment, ensure that the ambient temperature is not lower than 22°C.
2. Dispose of used tips and liquid waste according to the regulations.
3. After PCR amplification, wear disposable gloves to remove the PCR tubes or plates. Check whether the caps or seals are tightly closed and whether the walls are cracked. Discard in the designated area without opening the caps.
4. During the process of separating magnetic particles using the magnetic stand, rotate the tubes slowly to accelerate the aggregation of magnetic particles.
5. During DNA washing and eluting, check and briefly spin down the tube to collect any magnetic particles and elution buffer remaining on the tube cap or wall.
6. When removing residual ethanol, avoid over-drying the magnetic particles, as this may result in incomplete elution.
7. Perform DNA detection on the same day after DNA extraction to ensure accuracy.
8. Before starting the rHCDpurify® program, confirm the 96-well deep plate and the sleeves are properly secured.
9. Before or after the rHCDpurify® program running, UV sterilize the machine for at least 15 minutes and clean the inner walls with 75% ethanol wipes. The minimum interval between two extractions should be longer than 30 minutes.

Revision	Date	Description
A/0	20 Jul. 2026	Basic Version of User Guide.

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■ **Support & Contact**

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